Study: Goldman et al. (2012)

Quality: High (11 points)					
D 2	E 1	O 2	C 2	A 2	B 2

Goldman SM, Kamel F, Ross GW, Bhudhikanok GS, Hoppin JA, Korell M, Marras C, Meng C, Umbach DM, Kasten M, Chade AR, Comyns K, Richards MB, Sandler DP, Blair A, Langston JW, Tanner CM. Genetic Modification of the Association of Paraquat and Parkinson's Disease. Mov Disord. 2012, 27(13): 1652–1658.

STUDY SUMMARY

Study Overview

Goldman et al. conducted a high-quality case-control study nested in the Agricultural Health Study, a prospective cohort of licensed pesticide applicators and their spouses residing in two US states, Iowa and North Carolina. The objective of their investigation was to evaluate whether the risk of Parkinson's disease associated with paraquat exposure is modified by polymorphisms in the genes encoding for glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1). Participants in the underlying cohort were originally enrolled between 1993 and 1997. To assess gene-exposure interaction, the investigators genotyped 87 cases of Parkinson's disease and 343 controls matched on age, gender, and state of residence. Exposure to paraquat was either self-reported or reported by a proxy respondent and, for the interaction analysis, characterized as either "ever" versus "never" exposed. Years of lifetime paraquat use was also assessed and stratified into three categories: "never", used less than the median of 4 years, or used more than the median. Parkinson's disease diagnoses were determined by consensus of two movement disorder specialists using pre-established criteria. After adjustment for state of residence, age, and smoking, Parkinson's disease risk was significantly associated with report of "ever" using paraquat, relative to "never" use (OR: 2.6 ; 95% CI: 1.3-5.0). The investigators found that Glutathione S-transferase T1 (GSTT1) genotype was a statistically significant modifier of the relative odds of Parkinson's disease comparing paraquat-exposed and non-exposed study participants (p-interaction: 0.027). Paraquat-exposed male participants with functional GSTT1 had only a slightly increased odds of Parkinson's disease, relative to non-exposed male participants with functional GSTT1 (OR: 1.5; 95% CI: 0.6-3.6). However, Paraquat-exposed male participants with the homozygous deletion of GSTT1 had an 11-fold increased odds of Parkinson's disease, relative to non-exposed male participants with homozygous deletion of GSTT1 (OR: 11.1; 95% CI: 3.0-44.6). A similar interaction between paraquat exposure and glutathione S-transferase M1 (GSTM1) genotype was not observed. The authors concluded that, although replication of their findings is needed, individuals lacking glutathione S-transferase T1 (GSTT1) may have particularly high risk of Parkinson's disease risk associated with paraquat exposure.among.

Study Details

Study Participants. The base population for this nested case-control study consisted of participants in the Agricultural Health Study. [Tanner et al. 2011] The Agricultural Health Study is a large prospective study (n = 84,739) of licensed pesticide applicators and their spouses recruited between 1993 to 1997 in Iowa and North Carolina. Potential Parkinson's disease cases were initially identified using a screening questionnaire, and confirmed by consensus of two movement disorder specialists after an in-person examination by neurologists. Potential control subjects were identified by stratified random sampling of all living, non-demented participants in the Agricultural Health Study not suspected of having Parkinson's disease (screened negative) and frequency matched to cases by age, gender, and state of residence (Iowa or North Carolina) at a ratio of approximately three controls per case.

Exposure Measurement. History of exposure to paraquat and other non-genetic risk factors was ascertained primarily by self-report during a structured, computer-assisted telephone interview conducted by trained interviewers. Twenty-one cases and 52 control subjects, all men, reported ever mixing or applying paraquat. In a minority of instances (for 15 cases and 3 control subjects), a proxy respondent provided exposure information for study subjects who were unable to complete the interview because of death (afterblood collection), hearing or speech deficits, or cognitive impairment. Proxy informants for case and control subjects endorsed paraquat use with similar frequency (4 of 15 and 1 of 3, respectively). None of the female study subjects reported use of paraquat.

Genotyping. DNA was extracted from venous blood collected during in-home examinations. Genotyping was conducted by the genomics core at the University of California, San Francisco. The investigators tested for homozygous deletions of glutathione S-transferase T1 (GSTT1) and glutathione S-transferase M1 (GSTM1) using fragment-length multiplex polymerase chain reaction. The genotyping assay did not distinguish heterozygotes from non-null homozygotes.

Outcome Ascertainment.
An initial screening questionnaire was used by Agricultural Health Study investigators to identify cohort participants suspected to have Parkinson's disease. As part of the Farming and Movement Evaluation (FAME) study, neurologists assessed these suspected Parkinson's disease cases during an in-home visit. These assessments included a standardized neurological history, and an examination. Parkinson's disease diagnoses were determined by consensus of two movement disorder specialists using all available information, including findings from the in-home assessment and medical records, and by applying National Institute of Neurological Disorders and Stroke/UK Brain Bank criteria. [Gelb et al. 1999] The investigators did not exclude prevalent cases of Parkinson's disease.

Methods of Analysis. Multivariate logistic regression modeling was used to estimate unadjusted and covariate-adjusted odds ratios for self-reported paraquat exposure and Parkinson's disease, and for the evaluation of multiplicative paraquat exposure effect modification by polymorphisms in the genes encoding glutathione S-transferase M1 (GSTM1) and T1 (GSTT1). Because none of the female study subjects reported paraquat use, the analyses of gene-exposure interaction were conducted only among male subjects.

Confounders Considered. In addition to the matching factors (gender, age, state of residence), the investigators adjusted for smoking history () potential confounding by ethnicity (non-Hispanic Caucasian versus "other") and cigarette smoking (cumulative pack-years).

Effect Measure and Point Estimates. Results are presented as odds ratios and corresponding 95% confidence intervals. Adjusted for age, state of residence, and cigarette smoking, the odds of Parkinson's disease among subjects reporting "ever" paraquat exposure was 1.7 times that of subjects who reported "never" having had paraquat exposure (95% CI: 0.9-3.2). No females reported use of paraquat. Parkinson's disease odds increased with increasing levels of reported total years of lifetime paraquat use: Relative to those reporting never having used paraquat, the odds ratio among those reporting paraquat use less than or equal to the median of 4 years was

2.5 (95% CI:1.1-5.8) and 3.1 (95% CI: 1.3-7.2) among subjects reporting use of paraquat greater than the median of 4 years.

Parkinson's disease odds were modestly increased among subjects with homozygous deletion of the gene encoding glutathione S-transferase T1, but the association was not statistically significant (OR for all subjects: 1.5 (95% CI: 0.9-2.6); OR among male subjects only: 1.7 (95% CI: 0.9-3.2)). Homozygous deletion of the gene encoding glutathione S-transferase M1 was associated with reduced odds of Parkinson's disease (OR for all subjects: 0.8 (95% CI: 0.5-1.3); OR among male subjects only: 0.5 (95% CI: 0.3-0.9)).

Glutathione S-transferase T1 (GSTT1) genotype was a statistically significant modifier of the relative odds of Parkinson's disease comparing paraquat-exposed and non-exposed study participants (p-interaction: 0.027). Paraquat-exposed male participants with functional GSTT1 had only a slightly increased odds of Parkinson's disease relative to non-exposed participants with functional GSTT1 (OR: 1.5; 95% CI: 0.6-3.6), while paraquat-exposed participants with the homozygous deletion of GSTT1 had an 11-fold increased odds of Parkinson's disease, relative to non-exposed male participants with functional GSTT1 (OR: 11.1; 95% CI: 3.0-44.6). A similar interaction between paraquat exposure and glutathione S-transferase M1 (GSTM1) genotype was not observed, and odds ratios for the corresponding analyses were not included in the report.

Strength and Limitations Discussed in the Paper

Strengths:
Strengths discussed in the paper include the use of an agricultural cohort with a relatively large number of paraquat-exposed subjects, the high quality of diagnosis ascertainment, and the completeness and reliability of the paraquat exposure ascertainment. The matched, nested case-control design and use of an internal control group with similar exposure opportunities as the cases and similar demographic and lifestyle characteristics, was also cited as a strength of the investigation.

Limitations:
Limitations discussed by the authors included the possibility of misclassification due to reliance on participant self-report for ascertainment of paraquat exposure history, the small number of individuals that both reported paraquat exposure and had the homozygous deletions of the genes encoding glutathione S-transferase T1 (n=15),the corresponding imprecision in estimation of joint effects, possible non-identifiability of paraquat-specific effects due to the large number of study subjects that were exposed to other pesticides in addition to paraquat, the possibility of survivor bias due to the inclusion of prevalent Parkinson's disease cases at enrollment in the parent cohort study, and potential for bias due to reliance on proxy informants for a larger proportion of case subjects relative to control subjects.

EVALUATION

In their well-conducted case-control study (Farming and Movement Evaluation, FAME) nested within the Agricultural Health Study population, Goldman et al. (2012) found no evidence of a significant positive association between paraquat use and Parkinson's disease among men with functional glutathione S-transferase T1 (GSTT1) genotype. However, they observed statistically significant effect modification of the association between paraquat use and Parkinson's disease by GSTT1 genotype; having observed evidence of a strong positive association between paraquat use and Parkinson's disease among men with homozygous deletion of GSTT1. The nested design used in this investigation is optimal estimation of relative risks associated with gene-by-environment interactions without requiring blood sampling and resource-intensive genotyping of the entire cohort. Because of the nested design and random sampling of controls from the underlying cohort, this case-control study is protected selection bias arising from selection of control subjects whose exposure distribution is not representative of the study base. Participation among subjects was not a reported issue in the study, though proxy informants were used to ascertain exposure information for a minority of study subjects. Though use of proxy informants for a larger proportion of case subjects than control subjects could introduce bias, the authors noted that similar proportions of proxy respondents for case and control subjects reported paraquat use, and, furthermore, estimated associations from models adjusted for respondent type were qualitatively similar to the primary findings presented in the report. The number of subjects upon which the primary finding of effect modification is based (i.e. participants with reported exposure to paraquat and homozygous deletion of GSTT1) was very small, comprised of only 15 participants. Non-differential misclassification of paraquat use was possible due to the reliance on self-reporting and proxy reporting of exposure. However, several design elements may serve to minimize the specter of errors in association estimates due to paraquat misclassification: First, paraquat exposure classification was based on complete lifetime occupational history, rather than response to a single question regarding use of paraquat. The broad categorization of exposure ("ever" versus "never") may minimize errors in the paraquat exposure assignment, at the expense of reduced statistical efficiency, but even small errors in exposure assignment could have substantial effects on the observed association, since so few study subjects were exposed, particularly after stratification by genetic profile. Finally, non-differential misclassification of paraquat exposure would most likely diminish the magnitude of observed odds ratios, and as such, is not likely to have induced false-positive findings.

REFERENCES CITED

Tanner CM, Kamel F, Ross GW, et al. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011; 119:866-872.

Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Arch Neurol. 1999; 56:33–39.